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Ablation of capsaicin-sensitive afferent nerves affects insulin response during an intravenous glucose tolerance test

Esther H.E.M. van de Wall^{a,*}, Dorte X. Gram^b, Jan H. Strubbe^a,
Anton J.W. Scheurink^a, Jaap M. Koolhaas^a

^a*Department of Animal Physiology, Biology, Biological Center, University of Groningen,
P.O. Box 14, 9750 AA Haren, Groningen, The Netherlands*

^b*Pharmacology Research 3, Novo Nordisk A/S, Maaloev, Denmark*

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Abstract

We investigated the role of sensory nerves in glucose tolerance in conscious Wistar rats neonatally treated with neurotoxin capsaicin or vehicle. Intravenous glucose tolerance tests (IVGTT, 150, 300 and 450 mg in 30 min) were performed to measure glucose tolerance, and glucose, insulin and glucagon levels were measured.

Higher glucose concentration resulted in a greater insulin response in both capsaicin- and vehicle-treated rats. However, glucose-stimulated insulin secretion was attenuated in capsaicin-treated animals, even though glucose levels did not differ. Glucagon levels did not differ between both groups. These results show that capsaicin-sensitive nerves are involved in glucose-stimulated insulin secretion, but are not directly involved in the regulation of blood glucose levels. Moreover, they suggest that capsaicin-sensitive nerves could be involved in the regulation of insulin sensitivity. We hypothesize that sensory afferents could play a role in the aetiology of pathologies where glucohomeostatic mechanisms are disturbed, as is in type 2 diabetes mellitus.

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Keywords: Afferent nerves; Blood; Homeostasis; Vagus

* Corresponding author. Tel.: +31 50 363 2337; fax: +31 50 363 2331.

E-mail address: E.H.E.M.van.de.Wall@rug.nl (E.H.E.M. van de Wall).

Introduction

Insulin secretion is regulated by both autonomic nerves and by humoral factors. This is reflected by the rich innervation of pancreatic islets by parasympathetic, sympathetic and sensory nerves (Ahren, 2000). Classical neurotransmitters harboured in cholinergic and adrenergic nerves are important for the control of islet hormone secretion and therefore for controlling glucose homeostasis (Shimazu, 1996; Ahren et al., 1986, 1997; Kvist-Reimer et al., 2002). The brain appears crucial for coordinating an adequate response to glucose load, hypoglycemia and exercise. However, ample evidence suggests that peripheral loci are also important in the initiation of such a response. Little information exists regarding the relative involvement of these peripheral primary afferents in glucose homeostatic regulatory mechanisms, despite the fact that the entire gastro-intestinal tract—including important glucose-sensing organs such as liver and pancreas—is innervated by vagal afferents (Berthoud and Neuhuber, 2000). The importance of sensory (afferent) nerves, as A δ and C-fibers, in the control of glucose homeostasis has been shown in a number of studies. Although their exact function is not yet established, it appears that after treatment with the neurotoxin capsaicin, which destroys small unmyelinated fibers (Holzer, 1991), animals show an improved glucose tolerance accompanied with an increased early insulin response after an intravenous glucose injection (Karlsson et al., 1994). This improved glucose tolerance after capsaicin treatment was also seen during an oral glucose tolerance test, although the insulin response was not affected (Guillot et al., 1996). These results could indicate that capsaicin-sensitive sensory nerves are involved in the regulation of insulin sensitivity. Supporting evidence was obtained by Zhou et al. (1990) and Koopmans et al. (1998), who found strong indications that neonatal capsaicin treatment leads to improved insulin sensitivity. However, so far the mechanism responsible for these effects remains unclear. Calcitonin gene-related peptide (CGRP), the main neuropeptide present in capsaicin-sensitive nerves, affects islet hormone secretion by antagonizing the effect of insulin in vitro (Pettersson and Ahren, 1990) as well as in vivo (Pettersson et al., 1986; Leighton et al., 1989; Choi et al., 1991). This might explain the observed effects on insulin sensitivity after capsaicin treatment. Modulation by sensory nerves can be due to a local effector loop, but can also be mediated via afferent vagal glucose sensors present in the portal liver system. These are responsible for reflex modulation of efferent pancreatic vagus nerve activity, which can be abolished by hepatic vagotomy (Okazaki et al., 1993).

The improved glucose tolerance after capsaicin deafferentation might be mediated by glucagon. Insulin and glucagon are considered to be the key regulatory hormones for glucose homeostasis (Jiang and Zhang, 2003). The absolute level of glucagon or the ratio of glucagon to insulin is often elevated in various forms of diabetes in both animal and human subjects (Burcelin et al., 1996; Unger, 1985). This indicates that glucagon plays a major role in the regulation of glucose homeostasis. Because of a lack of afferent input, capsaicin-treated animals might exhibit a modified efferent output, and therefore, an altered basal glucagon secretion in comparison with vehicle-treated animals. Despite evidence that capsaicin-sensitive sensory nerve fibers are involved in the regulation of insulin sensitivity, neither their importance in the detection of glucose, nor the underlying mechanisms, is fully understood. Therefore, as part of a series experiments aimed at this problem, the present study investigated whether afferent nerves are involved in the detection for glucose. We compared the responses of capsaicin-treated and their vehicle controls during an intravenous infusion with 3 different glucose concentrations, over a period of 10 min. Previous studies on intravenous glucose tolerance tests (IVGTT) in capsaicin-treated animals used a single glucose injection. This causes a short and acute un-physiological disturbance in glucose homeostasis and shows only the removal rate of glucose from the bloodstream. We used slow

infusion of glucose over 30 min which is a much more physiological method that also allows frequent stress-free blood sampling in conscious animals. Thus, an intravenous glucose infusion gives more information compared to a single intravenous glucose injection. The present design allows us to determine whether capsaicin-treated animals are able to respond in the same fine-tuned manner as vehicle-treated animals. In addition to glucose and insulin, we measured glucagon levels to determine whether plasma changes in this insulin antagonistic hormone might contribute to the observed effects.

Materials and methods

Animals and housing, capsaicin treatment, surgery

Housing

Twenty male Wistar rats, bred in our own laboratory were used and housed in climate-controlled rooms (22 ± 2 °C) under a 12 h:12 h light–dark cycle (lights on at 8:00 am). Food and water were available ad libitum, unless otherwise specified. Capsaicin- and vehicle-treated pups grew up separately to avoid selective maternal care—in litters of 5–9 pups, in the proportion 5–7 male, 2 female. After weaning, at the age of 23 days, rats were individually housed in clear plexiglass cages ($25 \times 25 \times 30$ cm).

Capsaicin treatment

Capsaicin (8-methyl-*N*-vallinyl 6 nonenamide, 50 mg/kg; Sigma Chemical, Netherlands) was given neonatally ($n = 10$) by a subcutaneous (s.c.) injection. This was done under 100% O₂ conditions to avoid hypoxia. Capsaicin was dissolved in a vehicle consisting of 10% ethanol (10%) and 5% cremophore–0.9% sodium chloride solution (90%). Vehicle solution was injected s.c. ($n = 10$) in control rats. Each animal was given the same 50 µl volume, which we chose based on an average weight of the pups of 8 g.

At injection, both groups did not differ in body weight (capsaicin 7.9 ± 0.09 g; control 7.9 ± 0.09 g). After treatment, body weight was measured weekly, but no significant differences were observed between groups throughout the experiment (see Fig. 1). One capsaicin animal died during the nursing

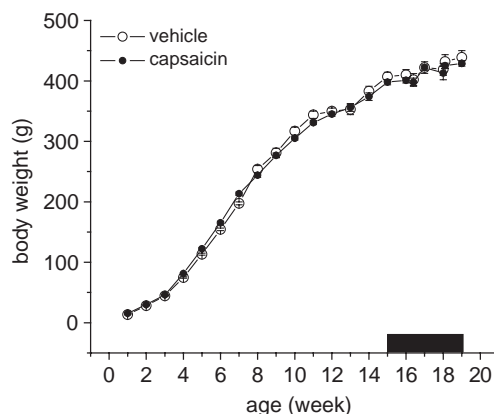


Fig. 1. Growth curve of capsaicin- and vehicle-treated animals. Black bar represents the experimental period. There are no significant differences between capsaicin- and vehicle-treated animals.

period, and 2 animals died during the experimental period, so we ended with a number of 5–7 animals per group.

An eye wipe response (0.1% capsaicin solution) was done at the age of 3 months in order to test the effectiveness of the capsaicin treatment. None of the capsaicin-treated animals responded to the test, so all animals were included in the experiment.

Surgery

Double heart catheters were implanted bilaterally in the vena jugularis under general anaesthesia with isoflurane/N₂/O₂; fentanyl (0.01 ml/100 g body weight) was given s.c. as post-surgical analgesia. Animals had at least 2 weeks of recovery before the start of experiments.

Experimental procedure and chemical determination

An intravenous glucose tolerance test (IVGTT) was performed. Each animal received an IVGTT concentration 3 times with at least 3 days in between. All treatments were randomly assigned. All experiments were performed in the light period between 12:00 and 1:00 pm. Animals were briefly food-deprived for 3 h before the experiment started.

To facilitate stress-free blood sampling, animals were connected to the tubing cannula at least half an hour before basal samples were taken. Glucose was infused during 30 min at a rate of 5, 10 or 15 mg/min, so each rat received a total glucose load of 150, 300 or 450 mg, respectively. Samples of 0.2 ml were taken at –11, –1, 1, 3, 5, 10, 15, 20, 25, 30, 40 and 50 min to measure glucose, insulin and glucagon concentrations. Blood and plasma samples were stored at –20 °C until analysis. Blood glucose levels were measured by Hoffman's ferrocyanide method; plasma level of insulin, and glucagon were measured by commercial radioimmunoassay kits (Linco Research; RI-13K, GL-32K, respectively).

Statistical analysis

Results are expressed as means \pm SEM. Repeated measurements analysis of variance (ANOVA) followed by LSD post hoc tests were used for statistical evaluation. Testing for significant differences were performed from the start of glucose infusion until end of infusion (0–30 min). Student's *t*-tests were used for unpaired observations. A *p*-value of <0.05 was considered significant.

Results

Fig. 1 shows that there are no significant differences in the growth curves of capsaicin- vs. vehicle-treated animals. Also, at start of the experiment, there were no differences in body weight between capsaicin- and vehicle-treated animals (398 ± 6.7 vs. 402 ± 10.1 g, NS).

Fig. 2 shows the changes in blood glucose levels (A), insulin (B) and glucagon (C) plasma over time during the 30 min 150, 300 and 450 mg i.v. glucose infusion. ANOVA for repeated measurements revealed that the 150, 300 and 450 mg glucose load induced significant changes in glucose ($F_{9, 108} = 27.82$; $F_{9, 90} = 92.63$; $F_{9, 81} = 85.27$, respectively, $p < 0.001$) and insulin ($F_{8, 96} = 7.39$; $F_{5, 55} = 5.72$; $F_{8, 112} = 32.99$, respectively, $p < 0.001$). Infusions of 150, 300 and 450 mg glucose did not induce significant changes in glucagon levels ($F_{9, 108} = 1.71$; $F_{8, 88} = 1.99$, $F_{9, 90} = 4.41$, respectively, NS).

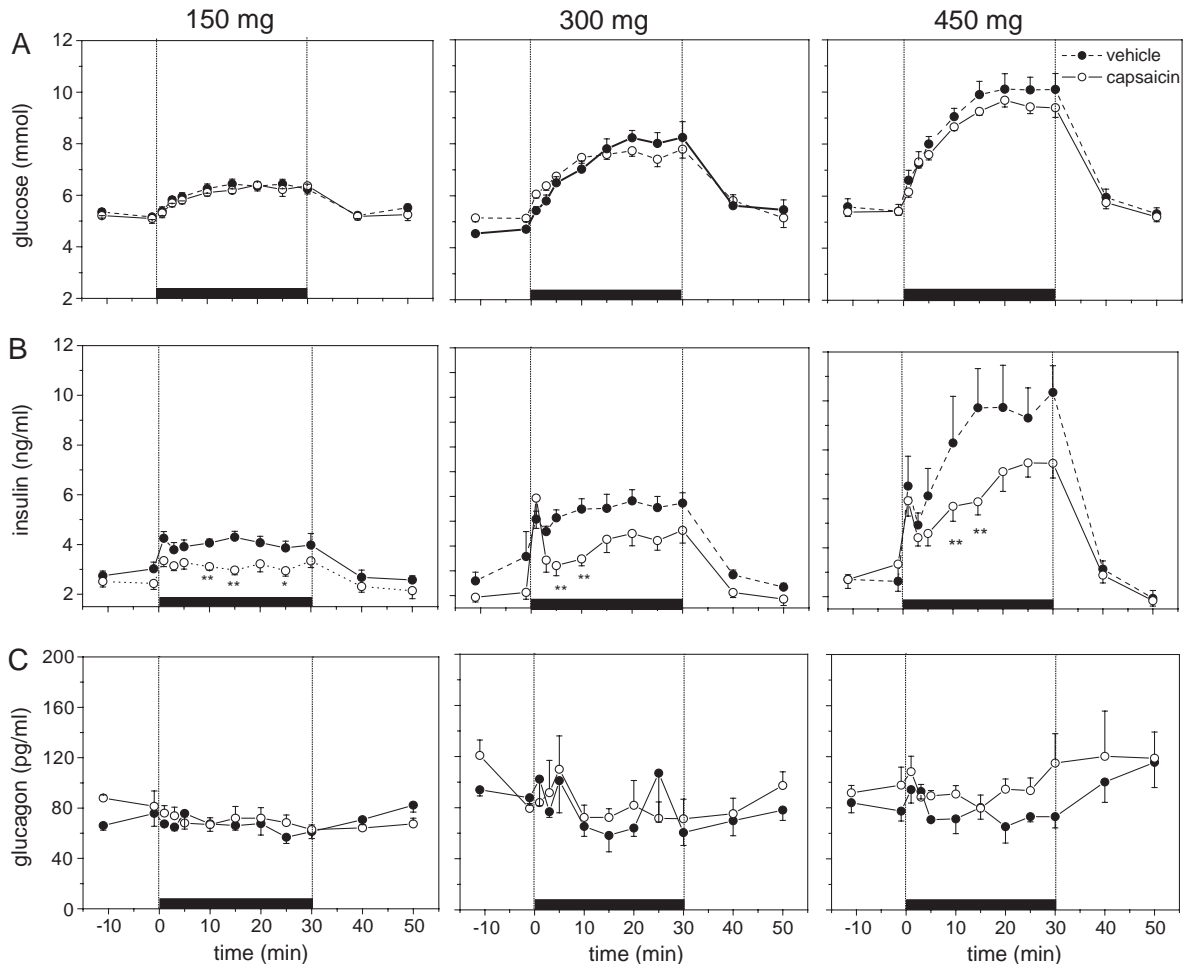


Fig. 2. Changes in plasma levels of glucose (A), insulin (B) and glucagon (C) before, during and after i.v. infusion of 150, 300 and 450 mg of glucose in capsaicin- and vehicle-treated animals. *, **Indicates significant difference between capsaicin- vs. vehicle-treated animal, $p < 0.05$ and $p < 0.01$, respectively.

For plasma glucose, there were no significant time \times group interactions for any of the 3 glucose infusions ($F_{9, 108} = 0.58$; $F_{9, 90} = 0.51$; $F_{9, 81} = 0.47$, respectively, NS). Also, there were no differences in glucose levels between capsaicin- and vehicle-treated animals during 150, 300 and 450 mg glucose infusions ($F_{1, 12} = 0.60$; $F_{1, 10} = 2.39$; $F_{1, 9} = 1.53$, respectively, NS). In contrast, plasma insulin levels did show significant effects of capsaicin treatment. Independent of the total glucose load (150, 300 and 450 mg), all capsaicin-treated rats had a significantly lower insulin response compared to their vehicle controls ($F_{1, 12} = 7.26$; $F_{1, 11} = 6.22$; $F_{1, 14} = 5.62$, respectively, $p < 0.05$). The early insulin response did not differ significantly between vehicle- and capsaicin-treated animals when the amounts of 450 mg (6.52 ± 1.22 vs. 5.92 ± 0.63 ng/ml, respectively, NS), 300 (5.08 ± 0.32 vs. 5.93 ± 1.03 ng/ml, NS) or 150 mg (4.25 ± 2.29 vs. 3.35 ± 0.24 ng/ml, respectively, NS) were infused. The differences between capsaicin- and vehicle-treated animals are particularly clear in the second phase of the insulin response. There was also no significant time \times group interaction for insulin levels during the 150 and 300 mg

Table 1

Basal values of glucose, insulin and glucagon before the start of infusion of 150, 300 and 450 mg glucose

Infusion	Glucose (mM)		Insulin (ng/ml)		Glucagon (pg/ml)	
	Vehicle	Capsaicin	Vehicle	Capsaicin	Vehicle	Capsaicin
5 mg/ml	5.3 ± 0.11	5.2 ± 0.14	2.9 ± 2.24	2.5 ± 0.22	75.4 ± 9.68	84.7 ± 6.84
10 mg/ml	5.1 ± 0.13	4.6 ± 0.09**	2.7 ± 0.27	2.0 ± 0.21	90.8 ± 5.03	123.1 ± 10.76
15 mg/ml	5.5 ± 0.27	5.8 ± 0.15	2.7 ± 0.11	2.5 ± 0.36	80.7 ± 4.11	94.9 ± 7.3

Values are averages ± SEM. Basal values are the average of basal measurements 11 and 1 min before start of the infusion.

** Significant difference between capsaicin- vs. vehicle-treated animals (2-tailed *t*-test; *p* < 0.01).

glucose infusion ($F_{8, 96}=1.49$; $F_{5, 55}=1.71$, respectively, NS). In contrast, the highest glucose load did result in significant time × group interaction for insulin plasma levels ($F_{1, 112}=2.79$, $p < 0.01$). Capsaicin- and vehicle-treated animals did not have different glucagon levels during the 150, 300 and 450 mg glucose infusion ($F_{1, 12}=0.65$; $F_{1, 6}=3.24$; $F_{1, 10}=1.91$, respectively, NS).

Table 1 shows the basal levels of glucose, insulin and glucagon. No significant differences were found between vehicle- and capsaicin-treated animals, except that basal glucose levels before 300 mg i.v. glucose infusion started was significantly lower in capsaicin-treated animals (5.1 ± 0.13 vs. 4.6 ± 0.09 mM, $p < 0.01$). However, since basal levels before the start of 150 mg or 450 mg i.v. glucose infusion did not differ between vehicle- and capsaicin-treated animals, the significance is of this result is unclear.

Discussion

The main result of this study is that neonatal capsaicin-treated male Wistar rats showed a diminished insulin response to an intravenous glucose infusion, while there was no difference in glucose tolerance when 150, 300 or 450 mg glucose loads were infused. Glucagon levels did not change during 150 and 450 mg glucose infusion in either deafferentated rats or their controls. There were also no significant differences between capsaicin- and vehicle-treated animals considering baseline plasma glucose and glucagon levels. This shows that a fine-tuned response to a glucose infusion does not depend (solely) on capsaicin-sensitive sensory nerves, since capsaicin- and vehicle-treated animals have similar glucose levels during an intravenous glucose infusion.

Our results are not in accordance with Karlsson et al. (1994) who found an improvement in glucose tolerance in capsaicin-treated animals after a glucose challenge. This improvement was accompanied by an enhancement of the early insulin response followed by lower insulin levels in capsaicin-treated animals (Karlsson et al., 1994). Yet, in our study, capsaicin-treated animals exhibited a reduced insulin response and there were no significant differences concerning the early insulin response. This discrepancy between studies may be due to the fact that Karlsson et al. (1994) gave only one bolus injection, whereas in our experiments the glucose infusion persisted for 30 min, this may partly have blunted the effect of this increased early insulin response. Furthermore, Karlsson et al. (1994) used female mice in their experiment which also might account for the observed differences with our results. It is known that the oestrous cycle has profound effects on glucose homeostasis (Bailey and Matty, 1972).

Since the lower insulin response was accompanied with normal glucose tolerance in our capsaicin-treated animals, results of the present experiments suggest that insulin sensitivity is increased. Results of

Guillot et al. (1996) also suggest this since animals had similar insulin responses but improved glucose tolerance after an OGTT. Earlier data of Zhou et al. (1990) and Koopmans et al. (1998) showed that after capsaicin treatment insulin sensitivity indeed improves. Although with the present set up, it remains unclear if the observed effects were due solely to increased insulin sensitivity, we do show that capsaicin-sensitive nerves are involved in insulin secretion. Another explanation could be that capsaicin-treated animals have an increased non-insulin-dependent glucose uptake, and as a result, a smaller insulin response might be required to normalize blood glucose levels.

It is unlikely that factors modulating insulin sensitivity, like body weight and nutritional state, could explain our results. It is known that a lower body weight can contribute to increased insulin sensitivity, and a period of fasting can influence the response of insulin (Strubbe and Prins, 1986). In our experiments, capsaicin treatment did not result in a different body weight compared to vehicle treatment. No major differences concerning nutritional state were expected, since all animals were briefly food-deprived for 3 h in the light period before the experiment started. We do not know if meal patterns differed between capsaicin- and vehicle-treated animals, which might result in different fasting periods before the experiment. However, such an explanation seems unlikely because this is usually associated with increased glucose levels (Strubbe and Prins, 1986), which were not observed.

The reduced insulin response might be a result of modifications of the responsiveness of the B-cells in capsaicin-treated animals. In vitro experiments on isolated islets exposed to capsaicin for several days showed no effect on B-cell function. Karlsson et al. (1994) and Koopmans et al. (1998) did not find altered islet secretory capacity in capsaicin-treated animals either. However, changes on islet level are reported, since systemic capsaicin treatment led to an increase in both whole pancreatic and islet blood flow, whereas fractional islet blood flow was decreased when compared with vehicle-treated rats (Carlsson et al., 1996). This may have resulted in a different insulin output as well.

Altered functioning of the catecholamine system could also contribute to the differences in glucohomeostatic mechanisms in capsaicin-treated animals as compared to controls. Catecholamines limit glucose utilization, increase glucose production from the liver, inhibit insulin secretion and stimulate glucagon secretion (Clutter et al., 1988). However, contradictory results are reported. Zhou et al. (1990) found increased catecholamine levels in response to insulin-induced hypoglycemia after neonatal capsaicin treatment, whereas Karlsson et al. (1994) did not find a significant effect on catecholamine levels. We cannot exclude the contribution of altered catecholamine plasma levels to the observed effects on insulin secretion. However, this explanation seems unlikely in view of the fact that no major changes in catecholamine levels are generally observed during an intravenous glucose tolerance test.

Glucagon is another insulin antagonistic hormone that might contribute to changes in plasma glucose levels. However, no major differences were found in plasma glucagon levels and could therefore not explain the present results. This result does not confirm the data of Karlsson et al. (1994), who observed an increased plasma glucagon level 10 min after one single glucose injection in capsaicin-treated animals. In addition, Karlsson et al. (1992) found a reduced glucagon response to a 2-deoxy-D-glucose injection in capsaicin-treated rats, while insulin response was not affected. They concluded that capsaicin-sensitive nerves are involved in the regulation of glucagon secretion. The present results show that glucose homeostatic regulation via afferent nerves can also be modulated without glucagon as mediator.

Nijima (1982) has shown that peripheral glucose-sensitive receptors exist in the liver. There are many data pointing clearly at the importance of vagal afferents for the detection of glucose and for

glucohomeostatic control (Zhou et al., 1990; Okazaki et al., 1993; Karlsson et al., 1994; Hevener et al., 1997, 2000; Koopmans et al., 1998). However, conflicting data exist as well. Jackson et al., (1997, 2000) and Cardin et al. (2001) raised some questions about the importance of vagal afferents for hypoglycemic detection, because they found that a functioning vagus nerve was not necessary for a complete counterregulatory hormone response to moderate hypoglycemia. Since substantial afferent innervation of the portohepatis ascends via the sympathetic nervous system, it could be that hepatic glucosensors communicate with the brain via these afferents (Jackson et al., 2000). However, it is as yet unclear whether the detection of hypoglycemic conditions is principally different from hyperglycemic conditions. Our results suggest that detection of hyperglycemia does not depend solely on capsaicin-sensitive nerves, since an infusion of different amounts of glucose results in a normoglycemic response accompanied with a fine-tuned insulin response. Our data do show that capsaicin-sensitive afferents are involved in insulin secretion in response to a certain glucose load. The modified insulin response in capsaicin-treated animals might reflect a change in efferent signalling as a result of the lack of afferent signalling; or, as suggested by Karlsson et al. (1994), it might be that capsaicin-sensitive nerves display their effects locally, by intrinsic nerves of the pancreas or by an afferent limb of a neural reflex regulation of glucose homeostasis. Since there are strong indications that insulin sensitivity has changed after capsaicin treatment, we suggest the hypothesis that capsaicin-sensitive sensory afferents are important in the (down) regulation of insulin sensitivity.

In summary, our results suggest that capsaicin-sensitive afferents are not primarily involved in glucose detection. We showed that neonatal capsaicin-treatment in male Wistar rats leads to a diminished insulin response to an intravenous glucose infusion. It appears that capsaicin-sensitive sensory nerve fibers are important in the regulation of glucose-stimulated insulin secretion and/or in the regulation of insulin sensitivity. Glucagon levels do not differ between capsaicin- and vehicle-treated animals and could therefore not explain the observed effects. We cannot exclude the possibility that present observations are due to changes on a secondary level after neonatal capsaicin treatment such as non-insulin-dependent glucose uptake, modified efferent output and/or local blood flow modifications on islet level. Since modifications of insulin secretion (Karlsson et al., 1994; this study), as well as on glucagon secretion (Karlsson et al., 1992) are reported, we suggest that capsaicin-sensitive sensory nerves might play a role in the onset and development of pathologies in glucohomeostatic mechanisms.

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